Bioelectrocatalysis at the Yeast Cell-Immobilized Electrode with Mediators

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1 INTRODUCTION
Recently we have reported on mediated bioelectrocatalysis using bacteria-immobilized electrodes. Intact cells of such bacteria, as Gluconobacter suboxidans immobilized on electrodes work as biocatalysts in the electrolytic oxidation of the substrates, glucose and ethanol, in the presence of electron transfer mediators between the electrodes and the immobilized cells of the bacterium. The catalytic function is due to the respiratory chain-linked enzymes which exist in the cytoplasmic membranes of bacteria belonged to procaryote. This may allows the substrates and the mediators to reach the enzyme with less difficulties. Yeast is an eucaryote and the respiratory enzymes exist in the mitochondrial particles within the cells. It is interesting to examine whether yeast as well as bacteria work as biocatalysts in the mediated bioelectrocatalysis reaction. In this paper we use baker's yeast and show that bioelectrocatalytic current is observed by the catalytic reaction of oxidoreductases within the yeast cells.

2 EXPERIMENTAL
Baker's yeast (Saccharomyces cerevisiae, IFO 2044) was cultured aerobically at 30 °C in a medium containing 0.3 % malt extract, 0.3 % yeast extract, 0.5 % polypeptone and 1 % glucose (pH 6.0). The yeast cells were harvested in the initial stationary phase of growth and washed with 0.1 mol/dm³ (M) potassium phosphate buffer (pH 6.0). The cell paste was suspended in the same buffer and the number of cells was determined by using a hemacytometer. The cell suspension was filtered through a cellulose nitrate membrane filter (Toyo Roshi Co., 1.0 μm pore size, 150 μm thickness) to adsorb on the filter and washed with the buffer. The filter (5 mm in diameter) was fixed at the carbon electrode (graphite/carbon composite electrode donated by Mitsubishi Pencil Co.) and covered with a nylon net. Electrochemical measurements were carried out with a three-electrode system in a deoxygenated buffer solution at 25 °C.

3 RESULTS AND DISCUSSION
The electrode with immobilized yeast cells was immersed in a 0.1 M phosphate buffer (pH 6.0) and the current at 0.2 V vs. Ag/AgCl (saturated KCl) of polarized potential was measured. The electrode produced an anodic current with the addition of 0.25 mM 2-methyl-1,4-naphthoquinone (Vitamin K₃, VK) as shown in Fig. 1. The current attained a steady-state after 20-30 min and gradually decreased after 2 h (data not shown). The steady-state current increased with increasing amount of immobilized cells (from $3.0 \times 10^4$ to $3.0 \times 10^6$ in the number of immobilized cells). The results indicated that VK was reduced by yeast cells and the reduced form of VK was oxidized electrochemically at the electrode. The electrode potential of 0.2 V was sufficiently positive to oxidize VK. The fact that there observed clear anodic current in the absence of
energy source such as glucose is interesting. In the case of acetic acid bacteria, similar anodic current was observed, but its magnitude was very small. Reduction of VK by the cells may be catalyzed by NADH:menadione oxidoreductase (EC 1.6.99.2) in the plasma membrane and cytosol of yeast, and NADH in the cytosol may be the electron donor. The reason for the slow current response may be that VK diffuses into and out of the cell through the plasma membrane. The decrease in the current magnitude observed after 2 h should be ascribed to the depletion of the concentration of NADH in the cells. The anodic current was also observed with the addition of 1,4-benzoquinone or Fe(CN)$_6^{3-}$, which has been shown to function as an electron acceptor of NADH:menadione oxidoreductase, though the current response was rather slow in the case of Fe(CN)$_6^{3-}$. Details of the bioelectrocatalytic behavior with addition of the mediators are under investigating.

When glucose was added to the solution containing VK, the anodic current increased further and attained a steady-state after 10 min (Fig. 1) as observed with acetic acid bacteria. The steady-state current with glucose also increased with increasing concentrations of glucose (Fig. 2). Similar current response was also observed with the addition of ethanol or lactate (Fig. 2), though the current magnitude was much smaller in the case of lactate. Above results suggest that the current magnitude depends on the concentration of NADH in the cells; glucose, ethanol and lactate (the substrates of NAD-dependent dehydrogenases) permeating into the cytosol cause an increase in the concentration of intracellular NADH by the catalysis of NAD-dependent dehydrogenases. The cell activity can be represented by the activity of NAD-dependent dehydrogenases. Thus, it is expected that the current is proportional to the cell activity. The electrode employing whole-cells of baker's yeast may be used for the determination of number of cells and the detection of cytotoxic compounds as well as bioreactor.

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REFERENCES